Anti-oxidation Activity of *Tabernaemontana divaricata* in Treating Diabetes Mellitus

CHAPTER 12

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SUMMARY

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insulin deficiency or resistance, which leads to severe complications such as neuropathy, nephropathy and cardiovascular diseases. Oxidative stress and inflammation play crucial roles in the pathophysiology of diabetes, which contributes to β-cell dysfunction and insulin resistance. Medicinal plants have gained significant attention for their potential in diabetes management due to their phytochemical composition and minimal side effects. *Tabernaemontana* (T.) *divaricate* is traditionally used for various medicinal purposes. Methanolic extract of *T. divaricata* effectively reduces oxidative stress and improves glycemic control, outperforming the hexane extract. This plant also has protective and regenerative effects on pancreatic β-cells. This suggests that *T. divaricata* holds potential as a natural therapeutic agent for diabetes management.

INTRODUCTION

Diabetes is a typical condition and its rate is significantly rising throughout the world. There are more than 171 million people globally having diabetes and this figure is probably going to dramatically increased by 2030. Due to the population aging, growth, obesity, unbalanced diet, inactive or lazy lifestyle, worldwide increment in diabetes will happen vast. (International Diabetes Federation, 2004). Diabetes mellitus (DM) is characterized by hyperglycemia (Karthikeyan et al., 2017). Hyperglycemia can cause changes in many metabolic pathways such as changes in the hexosamine biosynthetic pathway that results in increased O-glycation of protein intracellularly, increased formation of advanced glycation end products (AGEs) and changes in polyol pathway flux. The alterations of these metabolic pathways increase oxidative stress. In the polyol pathway, aldose reductase, the first enzyme is responsible for catalyzation of the process in which glucose is reduced to sorbitol. In the case of hyperglycemia, there is increased activity of aldol reductase that results in increased levels of sorbitol. Increased levels of sorbitol can disturb osmotic homeostasis resulting in cell damage. The second enzyme in the polyol pathway, sorbitol dehydrogenase, is responsible for the conversion of sorbitol to fructose. Thus, the use of antioxidants is considered effective in controlling the complications of diabetes (Gondi et al., 2017).

Besides oxidative stress, inflammation plays a main role in the pathophysiology of diabetes and its complications (Gothai et al., 2016). Inflammation can decrease the insulin secretion from the beta cells and increase the resistance of target cells against insulin. The cytokines can affect the beta cells directly or indirectly by adipocyte inflammation that can result in secretory dysfunction and increased apoptosis. This can result in lipotoxicity and glucotoxicity which can cause further inflammation and the cycle goes on. Inflammation has a main role in the progression from a pre-diabetic to a diabetic state (Agrawal and Kant, 2014). The inflammatory markers are also associated with diabetes related complications such as neuropathy, retinopathy, nephropathy and cardiovascular problems, etc. Thus, the use of anti-inflammatory compounds can be effective for the management of diabetes and its associated complications (Gothai et al., 2016).

Type 1 diabetes is characterized by the presence of inflammatory infiltrate in the islets of the pancreas. HLA and MHC genes are found to be associated with the susceptibility of the disease. The cell specific antibodies are present in the islet and there are alterations seen in the CD_4+ compartment of T-cells. Genetic susceptibility leads to an ineffective immune barrier and ineffective immune response. This results in the start of an autoimmune response that activates T lymphocytes and macrophages. The T lymphocytes then activate the CD4 receptor which then activates INF gamma that causes the destruction of beta cells. On the other hand, macrophages (the antigen presenting cells) activate TNF alpha and interleukin 1 which also causes beta cell destruction. If more than 90% of beta cells are destroyed it results in type 1 diabetes mellitus (Ozougwu *et al*., 2013). Type 2 diabetes accounts for 90% of cases of diabetes mellitus. Insulin deficiency due to beta cell dysfunction in insulin resistance by the tissues results in type

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2 diabetes. Obesity, sedentary lifestyle and old age population increase the risk of type 2 diabetes. Cardiovascular problems are often associated with type 2 diabetes and it is the major cause of morbidity in patients with type 2 diabetes. So, it is important to keep the lipid levels and blood pressure levels in check along with the monitoring of blood glucose levels (Chatterjee *et* al., 2017). In type 2 diabetes, there is no need to prevent ketoacidosis by insulin as it is not an autoimmune disorder. The genes responsible for type 2 diabetes are not identified, it may be due to heterogenicity of the genes. Under normal conditions, the blood glucose levels are kept within a narrow range, despite the changes in supply and demand, by insulin secretion and insulin sensitivity of the tissues. In type 2 diabetes, these mechanisms break down resulting in decreased levels of insulin due to beta cell dysfunction and impaired functioning of insulin due to insulin resistance in the tissues (Ozougwu *et al*., 2013).

There are many plants in nature that have therapeutic qualities. Numerous medications are made from these natural resources, and a large range of traditionally used medicinal plants are found all over the world (Kalaimagal & Umamaheswari, 2015). Medicinal plants are used extensively in Ayurveda, homeopathy, sidha, and folk medicine systems to treat human physiological ailments (Poddar et al., 2020). Around 80% of the world's population relies on plant medicines in their traditional health care systems, and the natural plant diversity of Earth is a rich supply of medicinal plants. Due to their minimal or nonexistent negative effects on humans, medicinal plants are used by millions of people worldwide for primary healthcare (Thombre et al., 2013; Sarkar et al., 2020). These therapeutic plants are all rich in phytochemical components. Determining a plant's therapeutic potential solely by looking at its phytochemical composition is a rather simple process (Poddar et al., 2020). Phytochemicals are derived from a variety of plant parts, including flowers, leaves, stems, fruits, and roots, to treat physical problems. The two types of phytochemicals used to describe this plant are primary metabolites, which include amino acids, proteins, carbohydrates, and chlorophyll, and secondary metabolites, which include phenols, glycosides, flavonoids, alkaloids, saponins, tannins, and terpenoids (Kalaimagal & Umamaheswari, 2015).

The evergreen shrub *Tabernamontana* (*T.*) *divaricata* is used as an ornamental plant. It is a member of the family Apocynaceae (Kalaimagal & Umamaheswari, 2015). Also called crepe jasmine. It is referred to as Thagar or Kath Mallika in West Bengal. This plant is widespread in the mangrove woodlands of Australia, Asia, Japan, China, and the nation of India. It is commonly found in the Upper Ganges plains, Garhwal, Visakhapatnam, and West Bengal (Khan, 2011). They are abundant in inhabited places, lawns, gardens, and by the sides of roadways. With its silver-gray bark, creamy white latex, glossy dark green foliage, and fragrant white blooms, the plant has a very appealing appearance. Because it contains a wide range of phytochemical components, including flavonoids, phenylpropanoids, terpenoids, steroids, alkaloids, and some plant enzymes, this plant is highly valued in traditional medicine (Ghosh et al., 2021).

BOTANICAL CLASSIFICATION OF *TABERNAEMONTANA DIVARICATA*

Divaricata is a member of the Tabermontanae tribe, the Apocynaceae family, the Plumeroidae subfamily, and the Tabernaemontana genus. The genus was given its name, Bergzabern, after J. Th. Mueller's birthplace; Bergzabern was latinized into Tabernaemontana. *T. Siamensis* is the basionym of *T. divaricata* (Van Beek et al., 1984) and Ervatamia siamensis is its homotypic synonym (Leeuwenberg, 1991). Typically having sweet-scented double flowers, the generic synonym Ervatamia is a garden plant that is extensively distributed in tropical areas. This genus contains over 100 species, most of which are found in tropical regions of the world like India, Thailand, Sri Lanka, Malaysia, Vietnam, Brazil and Egypt. Linnaeus published the first description of *T. divaricata* in 1753 (Van Beek et al., 1984).

"Crepe Jasmine," Tabernaemontana divaricata (syn. Ervatamia coronaria) is a glabrous, evergreen shrub with dichotomous branches that is primarily found in tropical climates. The plant can reach a maximum height of approximately six feet, and it produces lovely, fragrant white flowers with five petals arranged in little clusters at the tips of the stems. The leaves are huge, glossy, and have a deep green hue. They measure around 2 inches in width and 6 inches in length.

The plants have oval-shaped, simple, whole, opposite/sub-opposite, pinnately venated leaves. Their lanceolate to elliptic-oblong leaves have wavy, acuminate margins and are cuneate at the base. The leaves have a glossy, leathery texture and a deep green color. The plant's stem is smooth, hairless, dichotomously branched, rigid, erect, branching, thick, tuber-like, and has milky latex and silvery grey bark that is multi-trunked or clumping. It is also dark brownish in color. The plant's tap root system is its root. The firm, branching, silvery brownish root has no taste or smell (Khan, 2011).

The flowers have a flare bell shape, are white in color, are actinomorphic, have a sweet fragrance, and are arranged in pinwheels with solitary or few-flowered cymes in axils or terminals. The calyx has five lobes, which are imbricate, campanulate, and the corolla is salver-shaped with a tube that is dilated below the top. The stamens are five, included in the dilated portion of the tube, with short filaments and linear anthers; the carpels are two distinct, the style is long, the stigma is oblong, and there are many ovules. Fruits: The fruits are hard and infrequent, although they resemble pods or something similar. They have two follicles, are green on the exterior and orange-red inside, and taper into a narrow, curving beak. The plant produces a large number of uneven, minutely pitted, dull brown seeds that are encased in a crimson, pulpy aril (Khan, 2011). Flowers and fruit are all year long, but especially from July to September. A comprehensive examination of many plant species and their therapeutic principles has resulted in a contemporary worldwide reassessment of conventional medicine. Experimental results suggest that reactive oxygen species and free radicals may play a role in many different illnesses. Because they produce a lot of antioxidants to lessen the oxidative stress caused by

sunshine and oxygen, plants can be a source of new compounds with antioxidant activity.

Ethanolic extracts for this study because they include flavonoids, alkaloids, glycosides, saponins, tannins, and phenolic compounds. Perhaps they have components that actively scavenge free radicals (Jain et al., 2010). Eye infections are treated with the flower juice. The milky juice combined with oil is applied to the head to relieve eye pain; the root has an acidic and bitter flavor. Toothache can be relieved by chewing on the root, and wounds can be kept from inflaming by applying root paste. It has been used to treat fever, pain, and diarrhea in traditional Chinese, Ayurvedic, and Thai medicine (Kirtikar & Basu, 1918).

PHYTOCHEMISTRY

T. divaricata has been utilized for a variety of reasons, including traditional medicine. Previous reports have covered the phytochemistry as well as other chemical components from the leaves, stems, and roots. Alkaloids (Muzaffar A, 1985; Atta-ur-Rahman et al., 1986) and non-alkaloids includes terpenoids, steroids, flavonoids, phenylpropanoids, phenolic acids and enzymes (Dagino et al., 1991; Stevens et al., 1992). The 11 main classes of alkaloids of *T. divaricata* are, Eburan, Aspidospermatan, Corynanthean, Bis-indole, Vallesiachotaman, Strychnan, Vincosan, Plumeran, Ibogan, Tacaman and Miscellaneous (van Beek et al., 1984). By using a variety of techniques, including high performance liquid chromatography (HPLC), gas chromatography-mass spectrophotometry (GC-MS) and thin layer chromatography (TLC) atleast 66 alkaloids were isolated from *T. divaricata*. *T. divaricata* has also been shown to contain a number of non-alkaloidal components, including hydrocarbons, steroids, terpenoids, and enzymes.

Terpenoid-indole alkaloids and Enzymes

Formally, terpenoid-indole alkaloids are formed from a C10 unit of terpenoid origin (secologanin) and a unit of tryptamine, which is obtained via decarboxylation of tryptophan catalyzed by the enzyme tryptophan decarboxylase (TDC). The enzyme strictosidine synthase (SSS) catalyzes the stereospecific condensation of these two units. Numerous enzymes are involved in the metabolism and production of terpenoid compounds. Several investigations have elucidated the function of the enzymes that control terpenoids' production and metabolism in *T. divaricata* (van Beek et al., 1984). The five recognized enzymes that were discovered in *T. divaricata* cell suspension culture are the following (Fulton et al., 1994):

- Qualene 2,3 Oxide Cycloartenol Cyclase
- Isopentenyl Diphosphate Isomerase
- Squalene 2,3-Oxide Cyclase
- Squalene Synthetase
- Prenyl Transferase

In regulating the flow of carbon into the cytosolic microsomal pathway of terpenoid synthesis, these enzymes play a crucial regulatory role. Five enzymes, including strictosidine glucosidase, tryptophan decarboxylase, strictosidine synthase, geraniol 10 hydroxylase and isopentenyl pyrophosphate isomerase were discovered by Dagnino et al. (1995) from *T. divaricata* cell lines. Additionally, it has been proposed that these five enzymes are involved in the synthesis of *T. divaricata's* terpenoid indole alkaloids. Furthermore, *T. divaricata* cell suspension cultures were used to partially purify the enzyme strictosidine a-D-glucosidase (Luijendijk et al., 1996). Another non-alkaloidal enzyme, squalene synthase, was also partially isolated from a membrane-rich fraction obtained from suspension cultures of T. divaricata cells (Kroon & Threlfall, 1997). By using chromatography and the Western blotting technique, they identified the farnesyl diphosphate synthase enzyme from *T. divaricata* cultured cells (Ramos-Valdivia et al., 1998).

Secondary metabolites are a broad class of chemical compounds produced by many plant species that are not engaged in primary metabolism. Secondary metabolites are metabolic intermediates or products that are found in specific taxonomic groupings as differential products; they are not necessary for the growth and survival of the producing organism (Rhodes, 1994). A greater range of pathways than those seen in primary metabolism are used to biosynthesize them from one or more parent metabolites (Bennet & Bentley, 1989). The primary secondary metabolites with numerous physiological and pharmacological effects on live cells are terpenoids and alkaloids (Rhodes, 1994). Nonetheless, their production is typically limited to specific phases of the organism's development. A portion of the biosynthesises involves the phase-dependent synthesis of certain enzymes (Luckner & Nover, 1977). Consequently, the differentiation process of plants determines how secondary metabolites are expressed. It follows that the absence of secondary metabolite synthesis in the meristematic cells of whole plants is not surprising (Constabel, 1987). Furthermore, some research revealed that plant cell cultures may generate secondary metabolites if they cease to be meristematic and instead develop to a particular level of biochemical alteration and maturation (Rhodes, 1994). Thus, the production and metabolism of these secondary metabolites have been studied in a number of studies using cell cultures (Schripsema et al., 1994; Lucumi et al., 2001).

Non-alkaloids

Findings suggested that *T. divaricata* was an excellent scavenging mechanism to counteract air pollution. It was also discovered that several non-alkaloidal substances in T. divaricata, such as hydrocarbon, solid char, amino acid, and pyrolytic oil, have some advantageous properties. Indian *T. divaricata* stems and leaves contain pyrolytic oil and solid char that can be used to manufacture petroleum and ethanol, which can be used to make gasohol fuel, as demonstrated by (Sharma and Prasad, 1986). Additionally, hexane extract from *T. divaricata*'s old leaves, roots, flowers, and stems was hydrocarbon-rich (Behera et al., 1995).

Eight non-alkaloid chemicals, including a-amyrin acetate, lupeol acetate, a-amyrin lupeol, cycloartenol, b-sitosterol, campesterol, benzoic acid, and aurantiamide acetate have been reported from the root bark of *T. divaricata*. Their constituents

include plant metabolites such as phenolic acid and terpenoids, which demonstrate pharmacological characteristics including antioxidant and anti-inflammatory activity in vitro (Flores-Sanchez et al., 2002).

ANTIOXIDANT ACTIVITY AND ROLE IN TREATING DIABETES

Using an ethanolic solution of the diphenylpicryl hydrazine (DPPH) reagent, the antioxidant activity of the latex from *T. divericata* was ascertained. One stable free radical is DPPH. Antioxidants have the potential to donate hydrogen or scavenge free radicals, which has an impact on DPPH radical scavenging. Diphenylpicryl hydrazine is reduced and loses its violet color when a solution of DPPH is combined with a material that can donate a hydrogen atom. The test compounds' reactivity with a stable free radical scavenging activity is determined by the DPPH test. In the visible spectrum, DPPH has a significant absorption band at 517 nm. Free radical scavenger absorbs the odd electron when it pairs up, which decolorizes and lowers the DPPH solution. Pale yellow replaces the rich violet hue. Based on the findings, T.B. can be classified as having a moderate level of antioxidant activity and a measurably small amount of it (Raju & Rao, 2021). Thus it can be concluded that this plant has a measurable amount of antioxidant activity which is classified as a moderate level.

According to published research, carbonyl groups are in charge of scavenging free radicals (Nicholls & Budd, 2000). When oxygen reacts with specific compounds, free radicals—atoms or groups of atoms with an odd number of electrons—can be created. Highly reactive free radicals have the ability to ignite a chain reaction once they form. Their primary hazard is the harm they might cause when they interact with crucial biological constituents like DNA or cell membranes. This can lead to poor function or even cell death. The body's defense mechanism against free radical damage consists of antioxidants (Thomas, 2000). Free radicals are a byproduct of antioxidants; these radicals mate with unpaired electrons to eliminate the risk of gene modification, which can result in cancer(Patil et al., 2003). Professionals from a variety of medical systems as well as members of the scientific community from other fields have expressed interest in medicinal plants. As herbal medications are generally safe when used as directed, the World Health Organization (WHO) is actively pushing for the inclusion of these medications in national health care programs. Herbal medications are growing in popularity worldwide.

There has been a lot of interest in herbal treatments in the past few years for the treatment of various illnesses. One prospective source of medications is plants. The current study aims to ascertain the free radical scavenging activity of *T. divaricate* leaves in an effort to carry out the ongoing search for prospective free radical scavenging agents. The body's immune system is strengthened by the free radical scavenging qualities, aiding in the fight against cancer.

Based on experimental findings, a wide range of disorders may be related to reactive oxygen species and free radicals (D'Mello et al., 2000). Certain environmental factors and regular bodily cellular processes can produce free radicals. When these molecules receive an electric charge, one of their electrons is lost. Free radicals attempt to take an electron from or provide an electron to a nearby molecule in order to neutralize this charge. A chain reaction that can harm hundreds of molecules is started when the newly formed free radical seeks for another molecule and gives or withdraws an electron. This chain reaction is stopped by antioxidants. Certain antioxidants function as free radicals themselves, giving electrons to other harmful free radicals to help them stabilize (Matill, 1947). Other antioxidants combat free radical-forming molecules, eliminating them before they have a chance to set off a chain reaction that results in oxidative damage.

Free radical levels are regulated by antioxidants to prevent oxidative damage to the organism. For instance, the body's enzymes, like superoxide dimutase, combine with other substances to convert free radicals into compounds that are safe. Antioxidant vitamin C may guard against pancreatic, stomach, throat, and mouth cancers as well as cataracts (Matill, 1947). Additionally, it might stop LDL cholesterol from oxidizing, reducing the risk of heart disease. According to scientific literature, the flavonoids' and phenolic compounds' carbonyl groups were in charge of their ability to scavenge free radicals (Nicholls & Budd, 2000).The results of this study showed that the pharmacologically active chemicals found in *T. divaricata* Linn., including alkaloids, glycosides, saponins, tannins, flavonoids, and phenolic compounds, are what give the plant its superoxide scavenging activity.

Alloxan has been widely utilized to induce diabetic mellitus in animals due to its recognized specific cytotoxicity against pancreatic islet β cells. Reactive oxygen species (ROS) are produced by alloxan through a cyclic interaction with its reduction product, dialuric acid, when intracellular thiols are present. Free radicals produced in this redox reaction are what cause alloxan's beta cell toxicity. According to one study, alloxan does not cause diabetes in people. In the current investigation, we found that methanolic extract of Tabernaemontana divaricata (METD) can correct the metabolic disruptions brought on by alloxan-induced diabetes in rats (Kanthlal et al., 2014).

In order to comprehend the mechanism or mechanisms through which the methanolic extract of METD produces its hypoglycemic action, a number of biochemical markers were assessed in rats after a subchronic (21-day) course of treatment. The results of our investigation indicate that both METD and glibenclamide-treated rats showed a significant decrease in (i) blood glucose levels on various days, (ii) HbA1c levels and its HbA1c percentage, and (iii) serum cholesterol and TG levels. The extract also corrected changes in antioxidant indicators, including glutathione peroxidase, SOD, catalase, and lipid peroxidase. In diabetic control experimental rats, the decrease in total hemoglobin levels is directly correlated with elevated HbA1c levels. The most accurate marker and standard diagnostic parameter for determining the level of protein glycation in diabetes mellitus is HbA1c. Following oral treatment, experimental rats' HbA1c level was dramatically reduced by METD, maybe as a result of normoglycemic control mechanisms that also account for the rats' lower protein glycation condensation reactions.

Diabetic rats treated with alloxan have been observed to develop hypercholesterolemia and hypertriglyceridemia. Thus, it is possible to view the prominent hyperlipidemia that is associated with diabetes as a result of lipolytic hormones acting unchecked on fat deposits.

The METD-treated groups had lower levels of TG and cholesterol. It can be concluded that the effect of *T. divaricate* will be helpful in treating hyperlipidemia linked to diabetes. Increased LPO or indirect evidence of a decreased antioxidant reserve, such as SOD and catalase enzymes, in animal models, are the main causes of diabetes's elevated oxidative stress state. Diabetes induced by alloxan has been shown to exhibit elevated LPO and decreased levels of antioxidant enzymes. The body's defense mechanism against oxidative damage by free radicals (ROS) to the cell membrane and other components is comprised of antioxidant enzymes.

Because these enzymes are used in the inhibition or destruction of free radical species, there is a decrease in the serum concentration of total antioxidant enzymes in diabetic rats treated with alloxan. This suggests an imbalance in the synthesis of reactive oxygen species and antioxidant scavenging mechanisms. Additionally, our findings show that in diabetic rats, METD (100 and 200 mg/kg body weight) significantly increases antioxidant enzyme restoration and lowers LPO. Diabetes may cause a decrease in liver antioxidant enzyme levels due to an increase in hepatic cell utilization of these enzymes, which may be an attempt to counterbalance the increased generation of lipid peroxides. Moreover, there exists a direct correlation between insulin secretion and tissue damage caused by peroxides generated from lipoxygenase (Kanthlal et al., 2014).

ETHNOMEDICINAL USES OF *TABERNAEMONTANA DIVARICATA*

The wonderful and helpful shrub *T. divaricata* is employed as a traditional medicinal plant in homeopathic, ayurveda, and folklore treatment systems for mending numerous human problems across the globe. Numerous components of this herbaceous plant are used to cure a wide range of illnesses, including rabies, piles, rheumatism, epilepsy, asthma, diarrhea, stomach cancer and headaches. In addition, the plant is utilized as a purgative, anthelmintic, aphrodisiac, diuretic, brain, liver, and spleen tonic, as well as an antihypertensive and poison-avoidance remedy.

Anti-oxidative effects of *T. divaricata*

Many researchers have investigated its anti-oxidative properties utilizing the carbon tetrachloride (CCl4)-induced hepatotoxicity model (Mandal & Mukherji, 2001). The metabolite of CCl4, a free radical that causes lipids in the endoplasmic reticulum to peroxide and ultimately induce cell death, is responsible for hepatotoxicity. In an in vivo investigation, Gupta et al. (2004) discovered that methanol extract from *T. divaricata* leaves had a notable hepatoprotective effect by reducing lipid peroxidation and markedly raising the concentration of antioxidant agents like glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) in a dose-dependent manner. Moreover, *T. divaricata* is an excellent scavenging system.

Antifungal Activity

Ethanolic and petroleum ether extracts of *T. divaricata* were examined using the cup plate method to assess the plant's antifungal efficacy. In this instance, tests were conducted using various extract concentrations (5%, 10%, 15%, and 20%). Every Petri plate was filled with nutritional agar medium, and the plates were left to solidify. Microorganisms such as Aspergillus niger and Candida albicans were employed in this test, and they were swabbed on the Petri plate that contained medium. The four wells were prepared with a cork borer. These wells had varying concentrations of the sample. Next, all of the plates containing DMF and standard and control fluconazole were incubated for 48 hours at 28 °C. Thus, the plant extracts exhibited their antifungal effectiveness against Aspergillus niger and Candida albicans. Thus, the ethanol extract had a maximal inhibitory zone across the whole concentration range, whilst the petroleum ether extract and chloroform resulted in strong antifungal activity (Newall et al., 1996).

Anti-cancer Activity

The plant's hydroalcoholic decoction of petals was examined for its anticancer properties. The extract was made using the soxhlet extraction method. Here, hydroalcohol and petroleum ether were the solvents utilized. The experiment was conducted in an in vitro investigation using the human cancer cell line (HeLa). The MTT assay was employed to examine the inhibition of cell growth as well. The hydro-alcoholic decoction of *T. divaricata* flowers demonstrated a moderate level of anticancer activity, with an IC50 value over 100 μg/ml. Additional investigation is required to elucidate the plant's anticancer properties (Dantu et al., 2012).

Hepato-protective Activity

Rats were used to test the plant's hepatoprotective properties against DEN and Fe NTA-induced liver necrosis. 5-fluorouracil was the conventional medication, and *T. divaricata* ethanolic decoction was utilized at doses of 200 and 400 mg/kg body weight. Male Wistar Albino rats were given the sample and standard orally once a day for a period of 24 weeks. They received treatment with Fe NTA and the carcinogen DEN simultaneously. The plant extract significantly lowers blood levels of ALT, AST, uric acid, ALP and bilirubin parameters in rats receiving simultaneous treatment. It also raises the levels of marker enzymes in the liver. When compared to the treated group, the extract treatment resulted in a significant decrease in malondialdehyde levels and an increase in antioxidant component levels (Rehman et al., 2012). However, the outcomes were superior in every parameter when compared to rats given 200 mg/kg, 400 mg/kg, and 5 fluorouracil injections. Thus, our histological investigation verified the extract's protective activity against caused liver necrosis. As a result,

the plant has excellent hepatoprotective properties against the necrosis of liver cell (Poornima et al., 2012).

Nocicperception Activity

Using the model of acetic acid-induced writhing, the crude ethanolic extract of *T. divaricata* leaves demonstrated antinociceptive action in mice. At oral doses of 250 and 500 mg/kg body weight, the extract significantly reduced writhing in mice provoked with acetic acid. This was contrasted with a normal dosage of 25 mg/kg body weight of diclofenac sodium. According to the experiment, there was significant antinociceptive activity (Sharker et al., 2011; Rehman et al., 2012).

Anti-diabetic Activity

Rehman et al. (2012) checked blood glucose levels at intervals of 0, 2, 6, 12, and 16 hours after the extract was administered intraperitoneally at different doses of 300 and 400 mg/kg of body weight. The anti-hyperglycemic efficacy of the extract was compared with that of the common medication metformin. The extract was also put through a test for brine shrimp lethality. The extract's LC50 value was then 27.87µg/ml. Therefore, in comparison to conventional medications, the current findings showed that Tabernaemontana divaricata leaves have promise as an antidiabetic.

TOXICITY OF *TABERNAEMONTANA DIVARICATA*

Because *T. divaricata* exhibits several pharmacological actions, more toxicological research was required. Using the behavior screening test, Henriques and colleagues examined the toxicity of *T. divaricata* in mice given dosages of 150–200 mg/kg of alcoholic or aqueous extract (Henriques et al., 1996). They stated that there was no harm discovered at these dosages since the outcomes could not be distinguished from those of control animals. However, voacristine, one of *T. divaricata's* main alkaloids, showed dose-dependent cytostatic and cytotoxic effects on yeast cultures (Melo et al., 1986) Since the toxicity of *T. divaricata* has only been reported in small number of studies, more research on this topic will be necessary to fully comprehend its negative consequences.

CONCLUSION

Antioxidant biomarkers indicated that the methanolic has the potential to reverse the effects of alloxan-induced hyperglycemia but the methanolic extract of plant *T. divaricata* has good antioxidant and antidiabetic properties as compared to the hexane extract of *T. divaricata* as the methanolic extract reduced the oxidative stress significantly. Histopathological studies evaluated that the methanolic extract of *T. divaricata* regenerated the toxic potential of alloxan monohydrate.

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